

## 20.2 Fluorescent Ca<sup>2+</sup> Indicators Excited with UV Light

### Fura-2, Indo-1 and Derivatives

#### Fura-2 and Indo-1

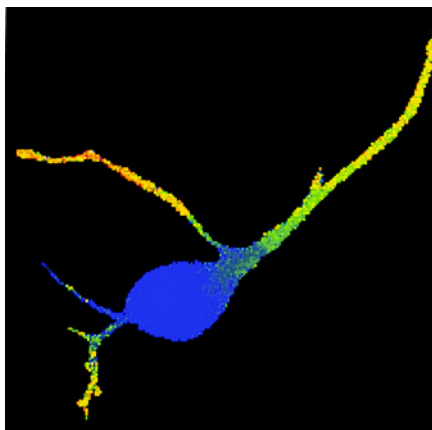
Fura-2 and indo-1 are UV light-excitable, ratiometric Ca<sup>2+</sup> indicators<sup>1</sup> that are generally considered to be interchangeable in most experiments. Fura-2 has become the dye of choice for ratio-imaging microscopy (Figure 20.2), in which it is more practical to change excitation wavelengths than emission wavelengths.<sup>2</sup> Upon binding Ca<sup>2+</sup>, fura-2 exhibits an absorption shift that can be observed by scanning the excitation spectrum between 300 and 400 nm, while monitoring the emission at ~510 nm (Figure 20.3). In contrast, indo-1 is the preferred dye for flow cytometry, where it is more practical to use a single laser for excitation — usually the 351–364 nm spectral lines of the argon-ion laser — and monitor two emissions.<sup>3,4</sup> The emission maximum of indo-1 shifts from ~475 nm in Ca<sup>2+</sup>-free medium to ~400 nm when the dye is saturated with Ca<sup>2+</sup> (Figure 20.4). Modern two-photon excitation imaging techniques used with fura-2 and indo-1<sup>5–8</sup> avoid the deleterious effects of conventional ultraviolet illumination on living specimens. Indo-1 may be less subject to compartmentalization than fura-2,<sup>9</sup> whereas fura-2 is more resistant to photobleaching than indo-1.<sup>10,11</sup> Both fura-2 and indo-1 exhibit K<sub>d</sub> values that are close to typical basal Ca<sup>2+</sup> levels in mammalian cells (~100 nM), and display high selectivity for Ca<sup>2+</sup> binding relative to Mg<sup>2+</sup>.<sup>12</sup> Nevertheless, Ca<sup>2+</sup> binding is discernibly perturbed by physiological levels of Mg<sup>2+</sup>; the K<sub>d</sub> for Ca<sup>2+</sup> of fura-2 is ~135 nM in Mg<sup>2+</sup>-free Ca<sup>2+</sup> buffers and ~224 nM in the presence of 1 mM Mg<sup>2+</sup>.<sup>12,13</sup> Fura-2 and indo-1 also exhibit high affinities for other divalent cations such as Zn<sup>2+</sup> and Mn<sup>2+</sup>, a property that is discussed further in Section 20.7.

The sodium and potassium salts of fura-2 (F-6799, F-1200; Figure 20.5) and potassium salt of indo-1 (I-1202, Figure 20.6) are cell-impermeant probes that can be delivered into cells by microinjection or using our Influx pinocytotic cell-loading reagent (I-14402, Section 20.8). Free acids of fura-2 and indo-1 can also be loaded into some plant cells at pH 4–5.<sup>14–18</sup> In addition, these salts are useful as standards for calibrating Ca<sup>2+</sup> measurements. Unlike the salt forms, the acetoxymethyl (AM) esters of fura-2 (Figure 20.7) and indo-1 can passively diffuse across cell membranes, enabling researchers to avoid the use of invasive loading techniques. Once inside the cell, these esters are cleaved by intracellular esterases to yield cell-impermeant fluorescent indicators (see Loading and Calibration of Intracellular Ion Indicators in Section 20.1). Molecular Probes offers fura-2 AM and indo-1 AM in 1 mg vials (F-1201, I-1203) or specially packaged in 20 vials of 50 µg each (F-1221, I-1223); the special packaging is recommended when small quantities of the dyes are to be used over a long period of time. We also provide stock solutions of fura-2 AM and indo-1 AM in anhydrous DMSO at 1 mg/mL (~1 mM; F-1225, I-1226). Our standard analytical specifications for fura-2 AM require ≥95% purity by HPLC. We also offer a special packaged FluoroPure grade of fura-2 AM that is specified to have ≥98% purity by HPLC (as a set of 20 vials, each containing 50 µg; F-14185). Dextran conjugates of fura and indo, as well as lipid analogs of fura for measuring near-membrane Ca<sup>2+</sup>, are described in Section 20.4.

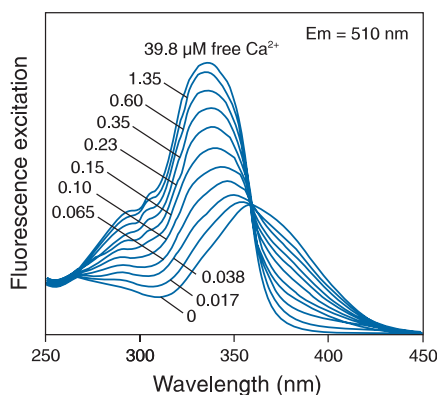
#### Fura-2 Calcium Imaging Calibration Kit

The Fura-2 Calcium Imaging Calibration Kit (F-6774), which is designed to facilitate rapid calibration and standardization of digital imaging microscopes,<sup>19,20</sup> contains the same 11 prediluted buffers as our Calcium Calibration Buffer Kit #2 (C-3009, Section 20.8). However, in this kit the buffers also include 50 µM fura-2, as well as 15 µm unstained polystyrene microspheres to act both as spacers that ensure uniform separation between the slide and the coverslip and as focusing aids. We also provide a twelfth buffer — identical to the 10 mM CaEGTA standard but lacking fura-2 — that serves as a control for background fluorescence. All of our Calcium Calibration Kits are described further in Section 20.8.

A product information sheet and extensive bibliographies are available for fura-2 and indo-1. Our bibliographic database currently contains over 4000 publications that cite the



**Figure 20.2** False-color image of free Ca<sup>2+</sup> concentration in a Purkinje neuron from embryonic mouse cerebellum. Neurons were grown in dispersed tissue culture for 12 days, loaded with the pentapotassium salt of fura-2 (F-1200) using a microelectrode and then challenged with *trans*-ACPD, an agonist of metabotropic glutamate receptors, in the absence of extracellular Ca<sup>2+</sup>. The composite image, which represents the ratio of images obtained with excitation at 340 nm and 380 nm, reveals the mobilization of internal Ca<sup>2+</sup> stores without contribution from Ca<sup>2+</sup> influx. Image contributed by D.J. Linden, Department of Neuroscience, Johns Hopkins University, and M. Smeyne and J.A. Connor, Roche Institute of Molecular Biology.



**Figure 20.3** Fluorescence excitation spectra of fura-2 (F-1200, F-6799) in solutions containing 0–39.8 µM free Ca<sup>2+</sup>.

*Our fura-4F, fura-5F, fura-6F and fura-FF Ca<sup>2+</sup> indicators have spectral responses to Ca<sup>2+</sup> that are virtually identical to those of fura-2, but with dissociation constants that permit measurement of higher levels of free Ca<sup>2+</sup> in cells.*

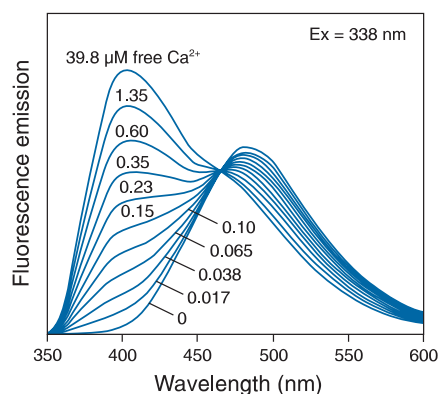
use of fura-2 AM for measuring intracellular free  $\text{Ca}^{2+}$  and over 1000 publications for indo-1 AM. For more information, contact our Technical Assistance Department.

### Bis-Fura-2: Brighter Signal with Lower Affinity for $\text{Ca}^{2+}$

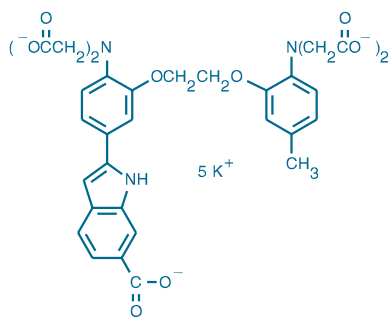
By linking two fura fluorophores with one BAPTA chelator (Figure 20.8), we have produced bis-fura-2, a  $\text{Ca}^{2+}$  indicator that exhibits approximately twice the absorptivity of fura-2. Bis-fura-2 has a  $K_d$  for  $\text{Ca}^{2+}$  of  $\sim 370$  nM and  $\sim 525$  nM in the absence and presence of 1 mM  $\text{Mg}^{2+}$ , respectively.<sup>21</sup> In other aspects, the quantum yield of bis-fura-2 and its spectral response to  $\text{Ca}^{2+}$  (Figure 20.9) are virtually identical to those of fura-2. Although

the difference between the  $K_d$  of fura-2 and bis-fura-2 for  $\text{Ca}^{2+}$  is small, the change in excitation ratio for bis-fura-2 in response to  $\text{Ca}^{2+}$  concentrations  $>500$  nM is larger than that of fura-2 (Figure 20.3); this difference can improve the dynamic range for  $\text{Ca}^{2+}$  measurements in cells.<sup>22</sup> Other potential advantages of bis-fura-2 include:

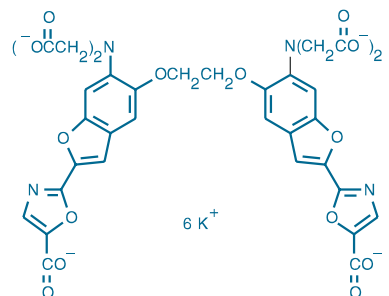
- Higher fluorescence output per indicator, which may allow the use of lower dye concentrations<sup>22</sup>
- Lower affinity for  $\text{Ca}^{2+}$ , which should decrease the buffering of intracellular  $\text{Ca}^{2+}$  and produce a faster response to  $\text{Ca}^{2+}$  spikes<sup>23</sup>
- An additional negative charge, which may facilitate dye retention



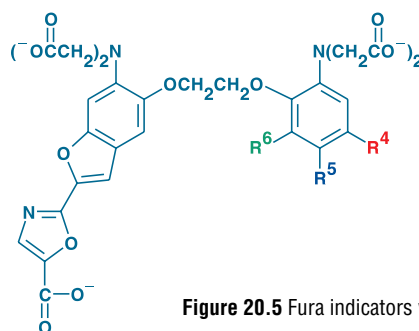
**Figure 20.4** Fluorescence emission spectra of indo-1 (I-1202) in solutions containing 0–39.8  $\mu\text{M}$  free  $\text{Ca}^{2+}$ .



**Figure 20.6** I-1202 indo-1, pentapotassium salt.

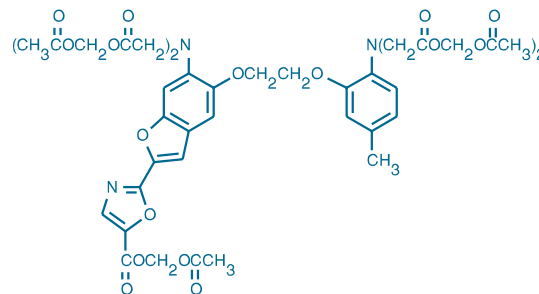


**Figure 20.8** B-6810 bis-fura-2, hexapotassium salt.

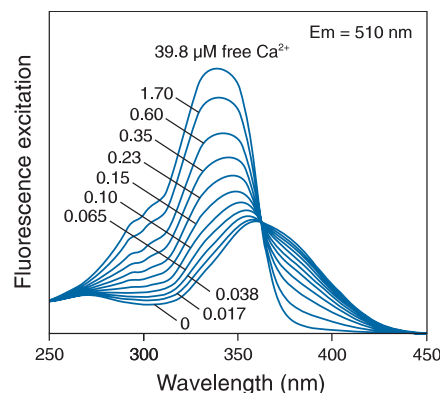


Indicator	$K_d(\text{Ca}^{2+})$	$R^4$	$R^5$	$R^6$
Fura-2	0.14 $\mu\text{M}$	H	$\text{CH}_3$	H
Fura-5F	0.40 $\mu\text{M}$	H	F	H
Fura-4F	0.77 $\mu\text{M}$	F	H	H
Fura-6F	5.30 $\mu\text{M}$	H	H	F
Fura-FF	5.50 $\mu\text{M}$	H	F	F

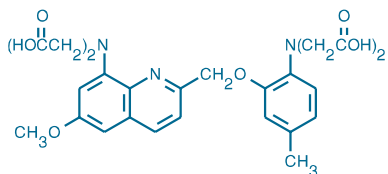
**Figure 20.5** Fura indicators with varying  $\text{Ca}^{2+}$  affinities.



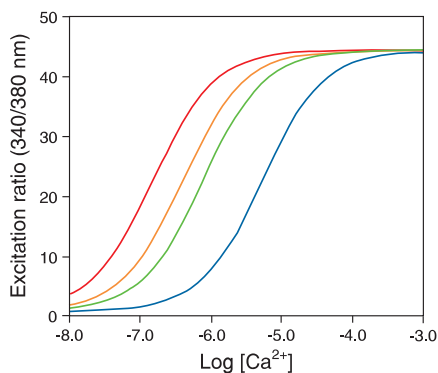
**Figure 20.7** F-1201 fura-2, AM.



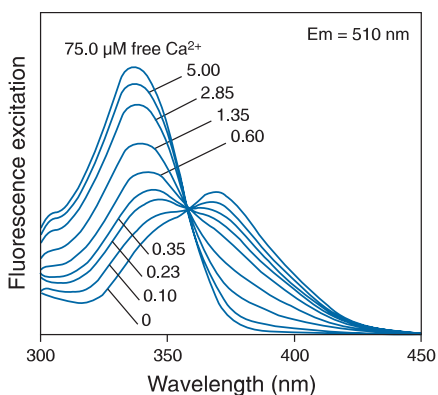
**Figure 20.9** Fluorescence excitation spectra of bis-fura-2 (B-6810) in solutions containing 0–39.8  $\mu\text{M}$  free  $\text{Ca}^{2+}$ .



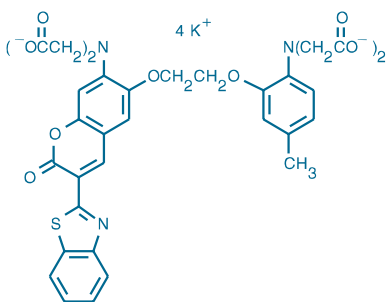
**Figure 20.10** Q-23918 quin-2, free acid.



**Figure 20.11** Fluorescence excitation ratio versus  $\text{Ca}^{2+}$  concentration curves for fura-2 (red), fura-5F (orange), fura-4F (green) and fura-6F (blue).



**Figure 20.12**  $\text{Ca}^{2+}$ -dependent fluorescence excitation spectra of fura-4F (F-14174).



**Figure 20.13** (B-6790) BTC, tetrapotassium salt.

The hexapotassium salt of bis-fura-2<sup>24–26</sup> (B-6810) is available for loading by micro-injection,<sup>27</sup> by diffusion from a patch-pipette<sup>28</sup> or using our Influx pinocytotic cell-loading reagent (I-14402, Section 20.8); we do not currently offer a membrane-permeant AM ester of bis-fura-2.

## Quin-2 and Quin-2 AM

Quin-2 belongs to the first generation of  $\text{Ca}^{2+}$  indicators developed by Tsien<sup>29</sup> (Figure 20.10). Quin-2 has lower absorptivity and quantum yield values than the fura-2, indo-1, fluo-3, fluo-4 and Calcium Green indicators and thus requires higher loading concentrations. The resulting high intracellular concentration of the indicator may buffer intracellular  $\text{Ca}^{2+}$  transients.<sup>30</sup> Quin-2 AM has been used to intentionally deplete cytosolic free  $\text{Ca}^{2+}$ <sup>31,32</sup> and to ensure unidirectional  $\text{Ca}^{2+}$  influx.<sup>33</sup> Measurement of cytosolic free  $\text{Ca}^{2+}$  with quin-2 has been thoroughly reviewed by Tsien and Pozzan.<sup>34</sup> Molecular Probes offers quin-2 as a high-purity, cell-impermeant free acid (Q-23918) and as its cell-permeant AM ester (Q-1289).

## Indicators with Intermediate Calcium-Binding Affinity

### Fura-4F, Fura-5F and Fura-6F

Calcium concentrations above 1  $\mu\text{M}$  produce almost complete binding saturation of fura-2 but very low fractional saturation of the low-affinity fura analog mag-fura-2 (M-1290, see below). To bridge this gap in the  $\text{Ca}^{2+}$  measurement range of fura-type indicators, we offer three additional ratiometric  $\text{Ca}^{2+}$  indicators — fura-4F (F-14174), fura-5F (F-14176) and fura-6F (F-14178) — and their corresponding membrane-permeant AM ester derivatives<sup>35</sup> (F-14175, F-14177, F-14179). Attachment of a single electron-withdrawing fluorine substituent at different positions on the BAPTA chelator moiety of fura-2 results in an increase of the  $K_d$  value<sup>36</sup> to ~770 nM, ~400 nM and 5.3  $\mu\text{M}$  for fura-4F, fura-5F and fura-6F, respectively (Figure 20.5). Except for the change in the  $\text{Ca}^{2+}$  concentration response range (Figure 20.11), the  $\text{Ca}^{2+}$ -dependent spectral shifts produced by fura-4F (Figure 20.12), fura-5F and fura-6F are essentially identical to those of fura-2 (Figure 20.3) and the probes use the same optical filter sets (Table 24.8).

### Fura-FF

Fura-FF is a difluorinated derivative of fura-2 (Figure 20.5) with a  $K_d$  value<sup>36</sup> of ~5.5  $\mu\text{M}$ .<sup>37–40</sup> Fura-FF has high selectivity for  $\text{Ca}^{2+}$ , a wide dynamic range and low pH sensitivity, making it an optimal low-affinity  $\text{Ca}^{2+}$  indicator for most imaging applications.<sup>37</sup> Although its spectroscopic characteristics are very similar to those of mag-fura-2, fura-FF has negligible  $\text{Mg}^{2+}$  sensitivity, making  $\text{Ca}^{2+}$  detection less susceptible to interference.<sup>38,39</sup> These properties have made fura-FF particularly useful for spatial and functional characterization of intracellular  $\text{Ca}^{2+}$  stores<sup>41–43</sup> and for tracking  $\text{Ca}^{2+}$  oscillations driven by the inositol 1,4,5-triphosphate receptor.<sup>44,45</sup> The low-affinity indicator fura-FF could detect NMDA- and kainate-induced neuronal  $\text{Ca}^{2+}$  fluxes that were not detectable with the higher-affinity indicator fura-2.<sup>46</sup> Fura-FF has also been used in combination with fura-2 and mag-fura-5 to compare the actions of  $\text{Sr}^{2+}$  and  $\text{Ca}^{2+}$  as mediators of synaptic transmission.<sup>38</sup> Fura-FF is available in water-soluble potassium salt form (F-14180) and as a membrane-permeant AM ester derivative (F-14181).

### Indo-5F

Indo-5F is an analog of indo-1 designed for measuring  $\text{Ca}^{2+}$  concentrations above 1  $\mu\text{M}$ . Like indo-1,<sup>38</sup> indo-5F exhibits  $\text{Ca}^{2+}$ -dependent dual-emission, making it suitable for ratio-metric detection by flow cytometry. Molecular Probes prepares indo-5F as a high-purity cell-impermeant potassium salt (I-23912) and as its cell-permeant AM ester (I-23913).

## Low-Affinity Calcium Indicators

### BTC

The coumarin benzothiazole-based  $\text{Ca}^{2+}$  indicator BTC (B-6790, Figure 20.13) and its cell-permeant derivative BTC AM (B-6791) were developed by Molecular Probes in

collaboration with Haralambos Katerinopoulos of the University of Crete.<sup>47,48</sup> This  $\text{Ca}^{2+}$  indicator exhibits a shift in excitation maximum from about 480 nm to 400 nm upon binding  $\text{Ca}^{2+}$  (Figure 20.14), permitting ratiometric measurements that are essentially independent of uneven dye loading, cell thickness, photobleaching and dye leakage. Its high selectivity and moderate affinity for  $\text{Ca}^{2+}$  ( $K_d \sim 7 \mu\text{M}$ ) allows accurate quantitation of high intracellular  $\text{Ca}^{2+}$  levels that are underestimated by fura-2 measurements.<sup>49,50</sup> Furthermore, because BTC is excited at longer wavelengths than the ratioable fura-2 and indo-1 indicators, cellular photodamage and autofluorescence may be less of a problem. When loaded into neurons as its AM ester, BTC exhibits little compartmentalization. However, prolonged excitation appears to cause conversion of the indicator to a calcium-insensitive form.<sup>50</sup>

BTC has been employed in investigations of  $\text{Ca}^{2+}$ -dependent exocytosis in pancreatic  $\beta$ -cells,<sup>51</sup> CHO fibroblasts<sup>52</sup> and pheochromocytoma cells.<sup>53,54</sup> Neuronal  $\text{Ca}^{2+}$  transients detected by the low-affinity  $\text{Ca}^{2+}$  indicators BTC and mag-fura-2 are significantly more rapid than those reported by the higher-affinity indicators fura-2 and Calcium Green-2.<sup>50,55</sup> BTC may also be useful as an indicator for  $\text{Zn}^{2+}$ .<sup>37</sup> A report by Papazoglou and co-workers describes the unusual use of the tetrapotassium salt of BTC to localize atherosclerotic plaque.<sup>56</sup>

### Mag-Fura-2, Mag-Fura-5 and Mag-Indo-1

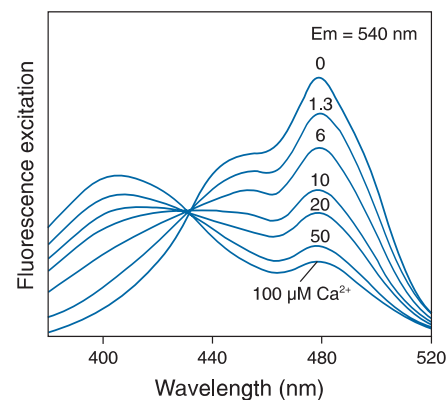
Mag-fura-2 (also called furaptra, Figure 20.62), mag-fura-5 and mag-indo-1 were originally designed to report intracellular  $\text{Mg}^{2+}$  levels (Section 20.6); however, these indicators actually have much higher affinity for  $\text{Ca}^{2+}$  than for  $\text{Mg}^{2+}$ . Although  $\text{Ca}^{2+}$  binding by these indicators may complicate analysis when they are employed to measure intracellular  $\text{Mg}^{2+}$ ,<sup>57,58</sup> their increased effective range and improved linearity for  $\text{Ca}^{2+}$  measurements has been exploited for measuring intracellular  $\text{Ca}^{2+}$  levels between 1  $\mu\text{M}$  and 100  $\mu\text{M}$ .<sup>37,59–61</sup>

The spectral shifts of mag-fura-2, mag-fura-5 and mag-indo-1 are very similar to those of fura-2 and indo-1 but occur at higher  $\text{Ca}^{2+}$  concentrations. Because the off-rates for  $\text{Ca}^{2+}$  binding of these indicators are faster than those of fura-2 and indo-1, these dyes have been used to monitor action potentials in skeletal muscle and nerve terminals with little or no kinetic delay<sup>62–65</sup> (Figure 20.15). The spectral properties, kinetics and selectivity of several of our low-affinity  $\text{Ca}^{2+}$  indicators have been reviewed by Zhao,<sup>66</sup> Hyrc<sup>37</sup> and their co-workers.

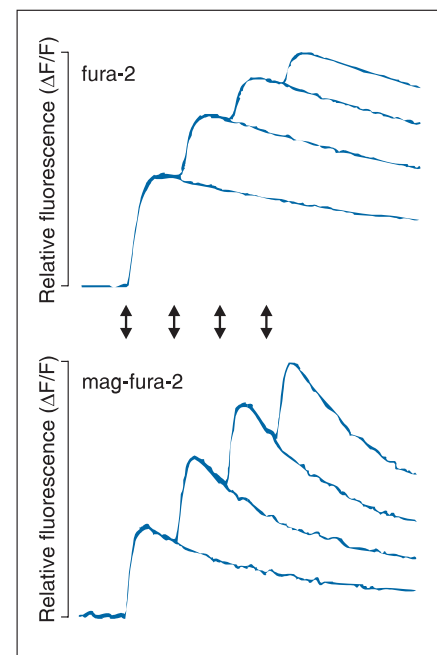
The moderate  $\text{Ca}^{2+}$  affinity of mag-fura-2 and the tendency of its acetoxymethyl (AM) ester to accumulate in subcellular compartments have proven useful for *in situ* monitoring of inositol 1,4,5-triphosphate-sensitive  $\text{Ca}^{2+}$  stores.<sup>67–71</sup> Mag-fura-2 has also been employed to follow  $\text{Ca}^{2+}$  transients in presynaptic nerve terminals,<sup>55,72–74</sup> gastric epithelial cells<sup>75</sup> and cultured myocytes.<sup>76</sup> Imaging of mag-fura-2 using a single excitation wavelength (420 nm) is reported to improve the detection of high-level  $\text{Ca}^{2+}$  transients in various cells, including Purkinje neurons and frog muscle.<sup>59,65</sup> Mag-indo-1 has been used to detect gonadotropin-releasing hormone-induced  $\text{Ca}^{2+}$  oscillations in gonadotropes<sup>77</sup> and to investigate the role of  $\text{Ca}^{2+}/\text{K}^+$  exchange in intracellular  $\text{Ca}^{2+}$  storage and release processes.<sup>78</sup> Measurements of  $\text{Ca}^{2+}$  currents in presynaptic boutons and granule cell parallel fibers with our mag-fura-5 and Magnesium Green indicators were shown to be superior to those made using fura-2.<sup>79,80</sup> Mag-fura-2, mag-fura-5 and mag-indo-1 are available as cell-impermeant potassium salts (M-1290, M-3103, M-1293) or as cell-permeant AM esters (M-1291, M-1292, M-3105, M-1295) and are accompanied by a detailed protocol.

## References

1. Fura-2, indo-1 and their structurally related analogs are licensed to Molecular Probes under US 4,603,209 and related patents; 2. *Methods Cell Biol* 56, 237 (1998); 3. *Methods* 21, 221 (2000); 4. *Methods Cell Biol* 41, 149 (1994); 5. *Biophys J* 75, 1669 (1998); 6. *Nat Neurosci* 3, 452 (2000); 7. *J Biol Chem* 273, 34961 (1998); 8. *J Microsc* 185, 9 (1997); 9. *Cell Calcium* 11, 487 (1990); 10. *Chem Biol* 3, 765 (1996); 11. *Am J Physiol* 253, C613 (1987); 12. *J Biol Chem* 260, 3440 (1985); 13. Measured at 37°C in 100 mM KCl, 10 mM MOPS, pH 7.0; 14. *Proc Natl Acad Sci U S A* 89, 3591 (1992); 15. *Plant Physiol* 93, 841 (1990); 16. *Plant Sci* 67, 125 (1990); 17. *Cell Calcium* 8, 455 (1987); 18. *Eur J Cell Biol* 46, 466 (1988); 19. *J Neurochem* 62, 890 (1994); 20. *Cell Calcium*



**Figure 20.14** Fluorescence excitation spectra of BTC (B-6790) in solutions containing 0–100  $\mu\text{M}$  free  $\text{Ca}^{2+}$ .



**Figure 20.15**  $\text{Ca}^{2+}$  transients evoked by trains of 1–4 action potentials in rat cerebellar granule cells detected by fura-2 (upper panel; F-1200) and mag-fura-2 (lower panel; M-1290). The stimulus pulses are 50 milliseconds apart (20 Hz); timing is indicated by the double-headed arrows. The amplitude of the transients detected by fura-2 decreases with each successive stimulus due to  $\text{Ca}^{2+}$  saturation. Mag-fura-2 avoids saturation due to its lower  $\text{Ca}^{2+}$  binding affinity ( $K_d$  for  $\text{Ca}^{2+} = 25 \mu\text{M}$ ), recording transients of approximately equal amplitude from successive action potentials. Adapted with permission from *Biophys J* 68, 2165 (1995).

*The full citations and, in most cases, links to PubMed for all references in this Handbook are available at our Web site ([www.probes.com/search](http://www.probes.com/search)).*

## References — continued

11, 75 (1990); **21.**  $K_d$  values for  $Ca^{2+}$  are determined at Molecular Probes at  $\sim 22^\circ C$  using our Calcium Calibration Buffer Kits; **22.** *Biophys J* 75, 1635 (1998); **23.** *Cell Calcium* 22, 255 (1997); **24.** *Neuron* 25, 229 (2000); **25.** *J Neurosci Res* 57, 906 (1999); **26.** *Neuron* 24, 727 (1999); **27.** *J Neurophysiol* 81, 2508 (1999); **28.** *Brain Res* 831, 113 (1999); **29.** *Biochemistry* 19, 2396 (1980); **30.** *J Biol Chem* 258, 4876 (1983); **31.** *J Biol Chem* 273, 8203 (1998); **32.** *Brain Res* 528, 48 (1990); **33.** *Biochemistry* 26, 6995 (1987); **34.** *Methods Enzymol* 172, 230 (1989); **35.** *Bioorg Med Chem Lett* 10, 1515 (2000); **36.** Measured at  $22^\circ C$  in 100 mM KCl, 10 mM MOPS, pH 7.2; **37.** *Cell Calcium* 27, 75 (2000); **38.** *Biophys J* 76, 2029 (1999); **39.** *Am J*

*Physiol* 266, C1313 (1994); **40.** US 5,516,911; **41.** *J Biol Chem* 276, 22461 (2001); **42.** *J Physiol* 529, 553 (2000); **43.** *Science* 275, 1643 (1997); **44.** *J Biol Chem* 274, 14157 (1999); **45.** *EMBO J* 16, 3533 (1997); **46.** *J Neurosci* 18, 7727 (1998); **47.** *Cell Calcium* 15, 190 (1994); **48.** US 5,501,980; **49.** *J Neurosci* 17, 6669 (1997); **50.** *Cell Calcium* 24, 165 (1998); **51.** *J Cell Biol* 138, 55 (1997); **52.** *J Biol Chem* 271, 17751 (1996); **53.** *J Physiol* 533, 627 (2001); **54.** *J Physiol* 494, 53 (1996); **55.** *Biophys J* 68, 2156 (1995); **56.** *J Photochem Photobiol B* 27, 81 (1995); **57.** *Anal Biochem* 290, 221 (2001); **58.** *Am J Physiol* 263, C300 (1992); **59.** *Pflügers Arch* 429, 587 (1995); **60.** *Proc Natl Acad Sci U S A* 90, 2598 (1993); **61.** *Neuron* 10, 21 (1993); **62.** *Proc*

*Natl Acad Sci U S A* 93, 8095 (1996); **63.** *J Physiol* 475, 319 (1994); **64.** *Biochem Biophys Res Commun* 177, 184 (1991); **65.** *J Gen Physiol* 97, 271 (1991); **66.** *Biophys J* 70, 896 (1996); **67.** *J Physiol* 530, 533 (2001); **68.** *J Cell Biol* 140, 325 (1998); **69.** *Cell Calcium* 20, 235 (1996); **70.** *EMBO J* 17, 1986 (1998); **71.** *Am J Physiol* 276, C426 (1999); **72.** *J Physiol* 527 Pt 1, 33 (2000); **73.** *Biophys J* 72, 1458 (1997); **74.** *Biophys J* 72, 637 (1997); **75.** *Am J Physiol* 267, G442 (1994); **76.** *Am J Physiol* 264, C1259 (1993); **77.** *Proc Natl Acad Sci U S A* 91, 9750 (1994); **78.** *Nature* 395, 908 (1998); **79.** *Biophys J* 74, 1549 (1998); **80.** *Biophys J* 73, 2476 (1997).

**Data Table — 20.2 Fluorescent  $Ca^{2+}$  Indicators Excited with UV Light**

Cat #	MW	Storage	Soluble	Low $Ca^{2+}$				High $Ca^{2+}$				Product	$K_d$	Notes
				Abs	EC	Em	Solvent	Abs	EC	Em	Solvent			
B-6790	844.03	L	pH >6	464	29,000	533	H <sub>2</sub> O	401	20,000	529	H <sub>2</sub> O/ $Ca^{2+}$	B-6790	7.0 $\mu$ M	1, 2, 3
B-6791	979.92	F,D,L	DMSO	433	39,000	504	MeOH							
B-6810	1007.14	D,L	pH >6	366	56,000	511	H <sub>2</sub> O	338	68,000	504	H <sub>2</sub> O/ $Ca^{2+}$		370 nM	1, 2, 4, 5
F-1200	832.00	D,L	pH >6	363	28,000	512	H <sub>2</sub> O	335	34,000	505	H <sub>2</sub> O/ $Ca^{2+}$		145 nM	1, 2, 4, 5
F-1201	1001.86	F,D,L	DMSO	370	31,000	476	EtOAc					F-1200		
F-1221	1001.86	F,D,L	DMSO	370	31,000	476	EtOAc					F-1200		
F-1225	1001.86	F,D,L	DMSO	370	31,000	476	EtOAc					F-1200		6
F-6799	751.45	D,L	pH >6	363	28,000	512	H <sub>2</sub> O	335	34,000	505	H <sub>2</sub> O/ $Ca^{2+}$		145 nM	1, 2, 4, 5
F-14174	835.96	D,L	pH >6	366	21,000	511	H <sub>2</sub> O	336	23,000	505	H <sub>2</sub> O/ $Ca^{2+}$		770 nM	1, 2, 4
F-14175	1005.82	F,D,L	DMSO	370	29,000	475	EtOAc					F-14174		
F-14176	835.96	D,L	pH >6	363	26,000	512	H <sub>2</sub> O	336	29,000	506	H <sub>2</sub> O/ $Ca^{2+}$		400 nM	1, 2, 4
F-14177	1005.82	F,D,L	DMSO	369	31,000	476	EtOAc					F-14176		
F-14178	835.96	D,L	pH >6	364	25,000	512	H <sub>2</sub> O	336	28,000	505	H <sub>2</sub> O/ $Ca^{2+}$		5.3 $\mu$ M	1, 2, 3
F-14179	1005.82	F,D,L	DMSO	369	30,000	477	EtOAc					F-14178		
F-14180	853.95	D,L	pH >6	364	25,000	510	H <sub>2</sub> O	335	28,000	506	H <sub>2</sub> O/ $Ca^{2+}$		5.5 $\mu$ M	1, 2, 3
F-14181	1023.82	F,D,L	DMSO	370	30,000	476	EtOAc					F-14180		
F-14185	1001.86	F,D,L	DMSO	370	31,000	476	EtOAc					F-1200		7
I-1202	840.06	D,L	pH >6	346	33,000	475	H <sub>2</sub> O	330	33,000	401	H <sub>2</sub> O/ $Ca^{2+}$		230 nM	1, 2, 4, 5
I-1203	1009.93	F,D,L	DMSO	356	39,000	478	MeOH					I-1202		
I-1223	1009.93	F,D,L	DMSO	356	39,000	478	MeOH					I-1202		
I-1226	1009.93	F,D,L	DMSO	356	39,000	478	MeOH					I-1202		6
I-23912	844.03	D,L	pH >6	347	20,000	475	H <sub>2</sub> O	331	20,000	412	H <sub>2</sub> O/ $Ca^{2+}$		470 nM	1, 2, 4
I-23913	1013.89	F,D,L	DMSO	355	39,000	480	MeOH					I-23912		
M-1290	586.68	L	pH >6	369	22,000	511	H <sub>2</sub> O	329	26,000	508	H <sub>2</sub> O/ $Ca^{2+}$		25 $\mu$ M	1, 2, 3
M-1291	722.57	F,D,L	DMSO	366	31,000	475	EtOAc					M-1290		
M-1292	722.57	F,D,L	DMSO	366	31,000	475	EtOAc					M-1290		
M-1293	594.74	L	pH >6	349	38,000	480	H <sub>2</sub> O	328	35,000	390	H <sub>2</sub> O/ $Ca^{2+}$		35 $\mu$ M	1, 2, 8, 9
M-1295	730.63	F,D,L	DMSO	354	37,000	472	MeOH					M-1293		
M-3103	600.70	L	pH >6	369	23,000	505	H <sub>2</sub> O	330	25,000	500	H <sub>2</sub> O/ $Ca^{2+}$		28 $\mu$ M	1, 2, 3
M-3105	736.60	F,D,L	DMSO	365	31,000	461	EtOAc					M-3103		
Q-1289	829.77	F,D,L	DMSO	348	4,000	446	MeOH					Q-23918		
Q-23918	541.51	D,L	pH >6	353	4,000	495	H <sub>2</sub> O	333	3,900	495	H <sub>2</sub> O/ $Ca^{2+}$		60 nM	1, 2, 10

For definitions of the contents of this data table, see "How to use This Book" on page viii.

### Notes

- Dissociation constants are known to vary considerably depending on the temperature, pH, ionic strength, viscosity, protein binding, presence of other ions (especially polyvalent ions), instrument setup and other factors. It is strongly recommended that these values be verified under user-specific experimental conditions.
- Spectra measured in aqueous buffers containing 10 mM EGTA (H<sub>2</sub>O) or a >10-fold excess of free  $Ca^{2+}$  relative to the  $K_d$  (H<sub>2</sub>O/ $Ca^{2+}$ ).
- Dissociation constant determined at Molecular Probes by fluorescence measurements in 100 mM KCl, 10 mM MOPS, pH 7.2, 0–1 mM free  $Ca^{2+}$  at  $22^\circ C$ .
- Dissociation constant determined at Molecular Probes by fluorescence measurements in 100 mM KCl, 10 mM MOPS, pH 7.2, 0–39.8  $\mu$ M free  $Ca^{2+}$  at  $22^\circ C$ .
- $K_d(Ca^{2+})$  for fura-2 and indo-1 from the original reference by Grynkiewicz, Poenie and Tsien (*J Biol Chem* 260, 3440 (1985)) are 224 nM and 250 nM, respectively, measured in 1 mM EGTA, 100 mM KCl, 1 mM free  $Mg^{2+}$ , 10 mM MOPS, pH 7.0 at  $37^\circ C$ . For bis-fura-2,  $K_d(Ca^{2+})$  in presence of  $Mg^{2+}$  is 525 nM (determined at Molecular Probes in 100 mM KCl, 10 mM MOPS, pH 7.2, 1 mM  $Mg^{2+}$  at  $22^\circ C$ ).
- This product is supplied as a ready-made solution in the solvent indicated under **Soluble**.
- This product is specified to equal or exceed 98% analytical purity by HPLC.
- The emission spectrum of  $Ca^{2+}$ -bound M-1293 excited at 340 nm has approximately equal peak intensities at  $\sim 390$  nm and  $\sim 480$  nm (*Biochemistry* 30, 702 (1991)).
- Dissociation constant determined in 100 mM KCl, 40 mM HEPES, pH 7.0 at  $22^\circ C$  (*Biochem Biophys Res Commun* 177, 184 (1991)).
- $K_d(Ca^{2+})$  for quin-2 was measured in 120 mM KCl, 20 mM NaCl, pH 7.05 at  $37^\circ C$ . Under the same conditions with addition of 1 mM  $Mg^{2+}$ ,  $K_d = 115$  nM (*Methods Enzymol* 172, 230 (1989)).

## Product List — 20.2 Fluorescent Ca<sup>2+</sup> Indicators Excited with UV Light

Cat #	Product Name	Unit Size
B-6810	bis-fura-2, hexapotassium salt *cell impermeant*	1 mg
B-6791	BTC, AM *cell permeant* *special packaging*	20 x 50 µg
B-6790	BTC, tetrapotassium salt *cell impermeant*	1 mg
F-1201	fura-2, AM *cell permeant*	1 mg
F-1221	fura-2, AM *cell permeant* *special packaging*	20 x 50 µg
F-1225	fura-2, AM *1 mM solution in dry DMSO* *cell permeant*	1 mL
F-14185	fura-2, AM *FluoroPure™ grade* *special packaging*	20 x 50 µg
F-6774	Fura-2 Calcium Imaging Calibration Kit *zero to 10 mM CaEGTA, 50 µM fura-2 (11 x 1 mL)*	1 kit
F-1200	fura-2, pentapotassium salt *cell impermeant*	1 mg
F-6799	fura-2, pentasodium salt *cell impermeant*	1 mg
F-14175	fura-4F, AM *cell permeant* *special packaging*	10 x 50 µg
F-14174	fura-4F, pentapotassium salt *cell impermeant*	500 µg
F-14177	fura-5F, AM *cell permeant* *special packaging*	10 x 50 µg
F-14176	fura-5F, pentapotassium salt *cell impermeant*	500 µg
F-14179	fura-6F, AM *cell permeant* *special packaging*	10 x 50 µg
F-14178	fura-6F, pentapotassium salt *cell impermeant*	500 µg
F-14181	fura-FF, AM *cell permeant* *special packaging*	10 x 50 µg
F-14180	fura-FF, pentapotassium salt *cell impermeant*	500 µg
I-1203	indo-1, AM *cell permeant*	1 mg
I-1223	indo-1, AM *cell permeant* *special packaging*	20 x 50 µg
I-1226	indo-1, AM *1 mM solution in dry DMSO* *cell permeant*	1 mL
I-1202	indo-1, pentapotassium salt *cell impermeant*	1 mg
I-23913	indo-5F, AM *cell permeant* *special packaging*	10 x 50 µg
I-23912	indo-5F, pentapotassium salt *cell impermeant*	500 µg
M-1291	mag-fura-2, AM *cell permeant*	1 mg
M-1292	mag-fura-2, AM *cell permeant* *special packaging*	20 x 50 µg
M-1290	mag-fura-2, tetrapotassium salt *cell impermeant*	1 mg
M-3105	mag-fura-5, AM *cell permeant* *special packaging*	20 x 50 µg
M-3103	mag-fura-5, tetrapotassium salt *cell impermeant*	1 mg
M-1295	mag-indo-1, AM *cell permeant* *special packaging*	20 x 50 µg
M-1293	mag-indo-1, tetrapotassium salt *cell impermeant*	1 mg
Q-1289	quin-2, AM *cell permeant* *special packaging*	10 x 1 mg
Q-23918	quin-2, free acid *cell impermeant*	5 mg

## 20.3 Fluorescent Ca<sup>2+</sup> Indicators Excited with Visible Light

Visible light–excitable Ca<sup>2+</sup> indicators offer several advantages over UV light–excitable indicators:

- Efficient excitation with most laser-based instrumentation, including confocal laser-scanning microscopes and flow cytometers
- Reduced interference from sample autofluorescence
- Less cellular photodamage and light scatter
- Stronger absorption by the dyes, which may permit the use of lower dye concentrations and therefore lower phototoxicity to live cells
- Compatibility with photoactivatable (“caged”) probes and other UV light–absorbing reagents, increasing options for multiparameter measurements
- Large Ca<sup>2+</sup>-dependent fluorescence intensity increases, resulting in sensitive detection of Ca<sup>2+</sup> transients

In many cases, large increases in fluorescence intensity upon mobilization of Ca<sup>2+</sup>, resulting in easy detection of Ca<sup>2+</sup> transients.

### Fluo-3, Fluo-4, Rhod-2 and Related Derivatives

#### Fluo-3

The Ca<sup>2+</sup> indicator fluo-3 (Figure 20.16) was developed by Tsien and colleagues for use with visible-light excitation sources<sup>1</sup> in flow cytometry and confocal laser-scanning microscopy<sup>2</sup> (Figure 20.17). More recently, fluo-3 imaging has been extended to include two-photon excitation techniques (Figure 20.18).<sup>3,4</sup> Fluo-3 imaging has revealed the spatial dynamics of many elementary processes in Ca<sup>2+</sup> signaling<sup>5–11</sup> (Figure 20.17, Figure 20.19). Since about 1996, fluo-3 has also been extensively used in cell-based high-throughput screening assays for drug discovery.<sup>12,13</sup> Fluo-3 is essentially nonfluorescent unless bound to Ca<sup>2+</sup> and exhibits a quantum yield at saturating Ca<sup>2+</sup> of ~0.14 (Figure 20.20). The intact acetoxymethyl (AM) ester derivative of fluo-3 is also nonfluorescent, unlike the AM esters of fura-2 and indo-1. The green-fluorescent emission (~525 nm) of Ca<sup>2+</sup>-bound fluo-3 is conventionally detected using optical filter sets (Table 24.8)